ALKALINE PHOSPHATASE FORMATION IN ESCHERICHIA COLI K10. I. AMINO ACID POOLS. II. CHEMICAL AND KINETIC PROPERTIES OF THE MULTIPLE FORMS

by

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CHAPTER V. SUMMARY

A. Comparison of the Amino Acid Pools of Wild-Type and Alkaline Phosphatase Regulatory Mutants.

The free amino acids in wild-type and alkaline phosphatase regulatory mutants of *E. coli* has been studied. Total amino acid content of cells grown in high or low phosphate revealed that lysine, ammonia, alanine, reduced glutathione and glutamic acid were the major components and comprised 80 - 85% of the total pool. Low phosphate growth conditions lowered the ammonia and raised the glutamic acid intracellular concentrations. When wild-type cells synthesized alkaline phosphatase linearly with time, amino acids in the pool did not fluctuate and stabilized at a constant level. This analysis of amino acid pool composition indicates that free amino acids are unrelated to the regulation of alkaline phosphatase.

B. Chemical and Kinetic Properties of the Multiple Forms.

1. Alkaline phosphatase from wild-type cells analyzed by DEAE-cellulose chromatography revealed three distinct and separable species of alkaline phosphatase. The enzyme profile derived from constitutive mutants (*R1*$^-$ and *R2*$^-$), results in one species of alkaline phosphatase.

2. Kinetic properties of the purified forms of alkaline phosphatase demonstrate a competitive type of inhibition with inorganic phosphate. Substrate specificity and pH optimum were similar in all species. The Km of wild-type enzyme ranged from 3.5 - 5.8 x 10^{-5} M, whereas, constitutive enzyme was from 0.7 - 2.6 x 10^{-5} M.

3. Denaturation by acid or urea and renaturation of wild-type enzyme results in 90 - 100% recovery of the original enzyme activity. Identical experiments with constitutive enzymes resulted in the recovery of 5 - 50% of the original enzyme activity. Reaction of urea and periodate on wild-type enzyme effects an apparent conversion to one major species.

4. The physico-chemical studies of enzymic forms of E. coli alkaline phosphatase indicate the multiple species to be conformers rather than isozymes.

5. It is suggested that alkaline phosphatase regulatory genes may subserve a role in determining the structural conformation of alkaline phosphatase.